Concurrent Activation of Dopamine D$_1$ and D$_2$ Receptors Is Required to Evoke Neural and Behavioral Phenotypes of Cocaine Sensitization

Christine Capper-Loup,[1] **Juan J. Canales,**[2] **Neena Kadaba,**[3] **and Ann M. Graybiel**[3]

[1]Department of Neurology, University Hospital, 3010 Bern, Switzerland, [2]Area of Psychobiology, University Jaume I, E-12080 Castellón, Spain, and [3]Department of Brain and Cognitive Sciences and the McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Repeated exposure to psychomotor stimulants produces a striking behavioral syndrome involving repetitive, stereotypic behaviors that occur if an additional exposure to the stimulant is experienced. The same stimulant exposure produces specific alterations in gene expression patterns in the striatum. To identify the dopamine receptor subtypes required for the parallel expression of these acquired neural and behavioral responses, we treated rats with different D$_1$-class and D$_2$-class dopamine receptor agonists and compared the responses of drug-naive rats with those of rats given previous intermittent treatment with cocaine. In rats exposed to repeated cocaine treatment, the effects of a subsequent challenge treatment with either a D$_1$-class agonist (SKF 81297) or a D$_2$-class agonist (quinpirole) were not significantly different from those observed in drug-naive animals: the drugs administered singly did not induce robust stereotyped motor behaviors nor produce significantly striosome-predominant expression of early genes in the striatum. In contrast, challenge treatment with the D$_1$-class and D$_2$-class agonists in combination led to marked and correlated increases in stereotypy and striosome-predominant gene expression in the striatum. Thus, immediately after repeated psychomotor stimulant exposure, only the concurrent activation of D$_1$ and D$_2$ receptor subclasses evoked expression of the neural and behavioral phenotypes acquired through repeated cocaine exposure. These findings suggest that D$_1$–D$_2$ dopamine receptor synergisms underlie the coordinate expression of both network-level changes in basal ganglia activation patterns and the repetitive and stereotypic motor response patterns characteristic of psychomotor stimulant sensitization.

Key words: stereotypy; gene expression; dopamine receptors; basal ganglia; striosomes; striatum

Psychomotor stimulants provoke the appearance of repetitive, stereotyped behaviors that become more pronounced with repeated exposure to the drugs. This augmented responsivity is a core feature of the behavioral sensitization syndrome that results from chronic drug exposure (Robinson and Becker, 1986; Kalivas and Stewart, 1991; Robinson et al., 1998) Studies in the last 30 years suggest that basal ganglia dysfunction is linked to the appearance of such stereotypies. Drug-induced stereotypies in experimental animals are sensitive to lesions of the nigrostriatal projection (Creese and Iversen, 1972; Fibiger et al., 1973), treatments with dopamine receptor antagonists (Iorio et al., 1983; Arnt, 1985; Arnt et al., 1987; Karler et al., 1995; White et al., 1998; Nestler et al., 2001), and mutations affecting dopaminergic transmission (Turski et al., 1984; Richter et al., 1999). In humans, stereotypies are clinical manifestations of a number of psychiatric, neurological, and developmental disorders (Manschreck, 1986; Antony et al., 1988; Ridley, 1994; Rosenberg et al., 1997; Graybiel and Rauch, 2000), and alterations in frontostriatal transmission are associated with conditions featuring compulsive and stereotyped behaviors (Cummings, 1995; Rosenberg et al., 1997; Volkow and Fowler, 2000; Goldstein et al., 2001; Graybiel and Canales, 2001).

How the basal ganglia and their dopamine-containing inputs contribute to the generation of stereotypy is unclear. One key to this problem might be the finding that stereotypy induced by psychomotor stimulants is strongly correlated with the degree of imbalance in the activation of the striosome and matrix compartments of the striatum and their associated basal ganglia output pathways. Cocaine- and amphetamine-induced stereotypy is predicted by the relative predominance of early gene activation of striatal neurons in striosomes, which are thought to modulate the activity of dopamine-containing cell groups in the midbrain (Canales and Graybiel, 2000). On this basis, we reasoned that, if predominant neuronal activation in striosomes were directly linked to motor repetition, such neural adaptation should be readily observed both after acute treatment with stereotypy-inducing drugs and immediately after the sensitization phase of stimulant treatment, without the requisite for an intervening period of drug withdrawal.

A second key finding is that concurrent stimulation of D$_1$-class and D$_2$-class dopamine receptors is required for dopaminergic stimulation to produce robust motor stereotypies and other specific motor behaviors (Clark and White, 1987; Arnt et al., 1988; Canales and Iversen, 1998; Berke and Hyman, 2000; Nestler et al., 2001). Similarly, in early gene assays, only such simultaneous activation of D$_1$-class and D$_2$-class dopamine receptors evokes a

striosome-predominant pattern of gene expression in the striatum (LaHoste et al., 1993; Wirtshafter and Asin, 1994). These findings suggest that the coordinate expression of sensitized neural and behavioral responses acquired through repeated exposure to psychomotor stimulants could depend on an interplay between subclasses of dopamine receptors.

To test these hypotheses, we measured, in cocaine-sensitized and drug-naive rats, the stereotypy induced by D$_1$-class, D$_2$-class, or D$_1$–D$_2$ agonist challenges and, in the same animals, measured the patterns of striatal early gene expression induced by these treatments immediately after cocaine sensitization. We found that combined D$_1$–D$_2$ agonist treatment was required to induce such alterations and that a strong positive correlation held between the degree of stereotypy and the degree of striosome-predominant gene expression elicited.

MATERIALS AND METHODS

Drug treatments. Male Sprague Dawley rats weighing 250–350 gm were treated according to procedures approved by the Massachusetts Institute of Technology Committee on Animal Care. Rats were kept under standard conditions of temperature and humidity with a 12 hr light/dark cycle (lights on at 7:00 A.M.), and they were handled daily for 2 d before drug treatments. Throughout, the animals were treated in groups of seven. Injections were given in the home cages. Drug-naive rats ($n = 56$) received single injections of the D$_1$-class dopamine receptor agonist SKF 81297 in doses of 1 or 3 mg/kg intraperitoneally (dissolved in 0.1% ascorbic acid), combined with the D$_2$-class dopamine receptor agonist quinpirole in doses of 1, 3, or 9 mg/kg intraperitoneally (dissolved in 0.9% saline) or were given injections of one of these agonists alone at a dose level of 3 mg/kg. Control animals received 0.9% saline only. After the injections, the behavior of the rats was observed by at least one observer blind to the treatment type (see below). In a second experiment, rats ($n = 49$) received repeated cocaine (or, for control, saline) treatments before challenge with SKF 81297 alone, quinpirole alone, or both agonists in combination. The cocaine (cocaine hydrochloride, 25 mg/kg, dissolved in saline) was administered intraperitoneally twice daily (10:00 A.M. and 5:00 P.M.) for 7 consecutive days. On day 8, each rat received a challenge with quinpirole (6 mg/kg, i.p.), SKF 81297 (6 mg/kg, i.p), quinpirole plus SKF 81297 (each at 3 mg/kg, i.p.), or saline. Systematic behavioral observations were made after each 10:00 A.M. treatment with cocaine or saline and after the final challenge with the dopamine receptor agonists or saline. At the end of the final observation period, the rats were deeply anesthetized with sodium pentobarbital (Nembutal; >25 mg/kg) and were perfused transcardially with 4% paraformaldehyde in 0.1 M NaKPO$_4$.

Behavioral analysis. The induction of stereotyped behaviors was assessed during 1 hr after the experimental treatments by following a standardized 10-point rating scale (1, undetectable; 2, very weak; 3, weak; 4, weak-to-moderate; 5, moderate; 6, moderate-to-strong; 7, strong; 8, intense; 9, very intense; 10, extreme) (Canales and Graybiel, 2000) modified from Creese and Iversen (1972).

Stereotypy ratings were computed for each animal and for each observation period by calculating the mean score across four behavioral dimensions ranging between 1 and 10 in severity. The four behavioral dimensions were repetitiveness (degree of switching between different behavioral responses, with the exclusion of feeding and drinking responses), frequency (degree of intensity with which a single motor response was emitted), duration (estimation of the length of time engaging in motor stereotypy), and spatial distribution (degree of spatial confinement of the motor response, with the exclusion of periods of sleep). Scores were based on these four estimates of the motor responses emitted during 1 min periods 20 and 50 min after treatment. The average of these values was recorded as the session score. Monitored responses included movements of the head and limbs, stereotyped sniffing, and directed oral stereotypies. Scoring was done by an observer blind to the treatments given, and extended observation periods and simultaneous observation by two or three observers blind to the treatments were performed periodically. In preliminary experiments, all ratings were made independently by two or three observers, and the scores were found to match closely.

Immunohistochemistry. Brains were cut at 24 μm on a sliding microtome, and sections through the caudoputamen were stained by dual-

antigen immunohistochemical protocols with antisera raised against either c-Fos (Ab-5; 1:40,000; Oncogene Sciences, Manhasset, NY) or JunB (1:10,000; gift from Dr. R. Bravo) and the C-terminal peptide of the μ-opioid receptor (MOR1) (1:20,000; DiaSorin, Stillwater MN), a marker for striosomes, as described previously (Canales and Graybiel, 2000). Briefly, after incubation with the primary antibody, sections were exposed to a biotinylated secondary antibody and to an avidin–biotin complex conjugated to HRP (ABC Kit; Vector Laboratories, Burlingame, CA). Specific double stains were obtained by exposure to DAB-H$_2$O$_2$ (MOR1) and to DAB-H$_2$O$_2$ with nickel (NiSO$_4$) intensification (c-Fos and JunB).

Data analysis. For each brain, two sections through the rostral caudoputamen were analyzed. A ~4 mm^2 area at the center of the caudoputamen was divided into medial and lateral halves, and the outlines of the MOR1-positive striosomes within these regions were drawn. Fos-positive nuclei were counted with a microscope attached to an image analysis system (Biocom, Les Ulis, France). The counting was done by an observer blind to the experimental treatments. The counting procedure permitted calculations, for each section, of the density of Fos-positive neurons in the μ-opioid receptor-rich striosomes and in the surrounding matrix. For each individual animal, average values for the two sections counted were used to calculate an index of the relative gene expression in striosomes compared with that in the matrix [index of striosome-to-matrix predominance (ISMP)] by taking the ratio between the density values of Fos-positive nuclei in the striosomes and in the matrix of the sampled regions (Canales and Graybiel, 2000). The gene expression data for each treatment group were analyzed statistically by means of ANOVA. Kruskal–Wallis and the Kolmogorov–Smirnov tests were used for analysis of the behavioral observations. Correlations between gene expression values and stereotypy scores were calculated by the method of Spearman (r_s values).

RESULTS

Coactivation of D$_1$-class and D$_2$-class dopamine receptors is required to induce strong motor stereotypies in normal and cocaine-pretreated animals

To establish baselines for the behavioral effects of the dopamine receptor agonists, we first measured the stereotypies induced by different single or combined doses of the D$_1$-class and D$_2$-class agonists. Confirming previous results (Arnt et al., 1987, 1988), acute treatment with combinations of the D$_1$-class and D$_2$-class receptor agonists induced oral stereotypies, including nibbling of the cage material, licking, and head-down sniffing (Fig. 1A). Treatment with quinpirole alone induced stereotypies of mild intensity, not reaching statistical significance compared with control values ($p = 0.063$). SKF 81297 treatment alone did not produce such responses but only nondirected behavioral activation. Stereotypy levels appeared to saturate at values of ~5 on the rating scale after the combined D$_1$–D$_2$ dopamine agonist treatments. Even the lowest doses of the two agonists (1 mg/kg each SKF 81297 and quinpirole), when given in combination, produced high stereotypy ratings, and higher doses of either or both did not produce additional increases in stereotyped behavior. These results suggest that significant levels of dopamine receptor-stimulated stereotypy depend on stimulation of dopamine receptors of both D$_1$ and D$_2$ subclasses and that even large doses of either agonist alone are ineffective in augmenting such stereotypic behavior. The lack of dose dependency of the combined D$_1$–D$_2$ agonist treatments for the expression of stereotypies contrasts with the capacity of apomorphine to evoke such behaviors in a dose-dependent manner (Canales and Graybiel, 2000), which could either be attributable to the differential D$_1$–D$_2$ affinity ratios of the drugs or to the fact that we did not test yet lower doses of the agonists.

Repeated administration of cocaine produced a progressive enhancement of stereotyped behavior over time, in accord with previous findings (Jung et al., 1999; Canales and Graybiel, 2000;
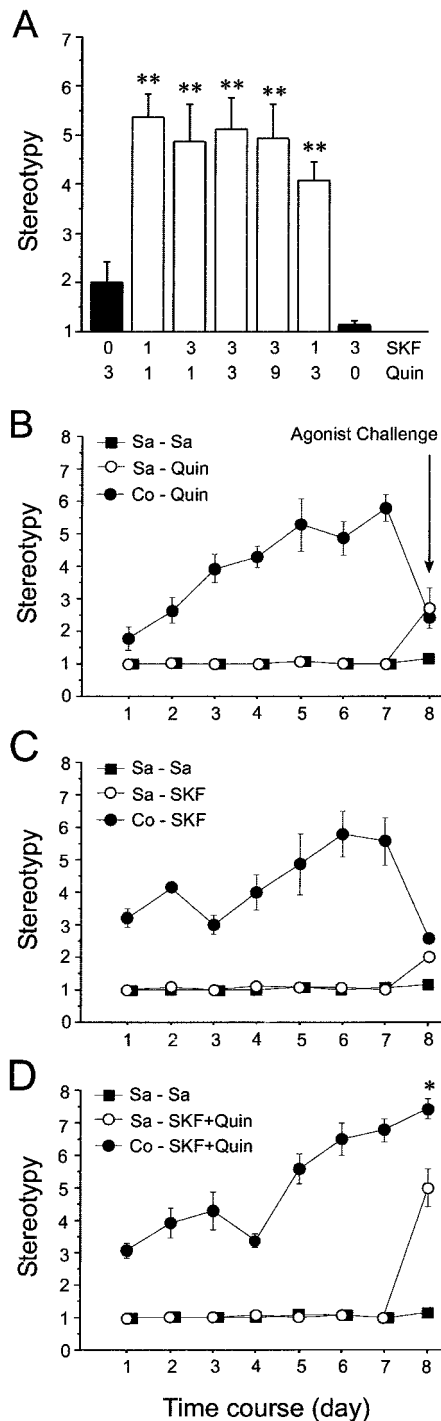
Figure 1. Acute treatment with a combination of dopamine D_1-class receptor agonist (SKF 81297) and dopamine D_2-class agonist (quinpirole), but not treatments with each class of dopamine agonist given alone, induces motor stereotypy. *A*, Stereotypy scores after acute administration of different dose combinations of SKF 81297 (0, 1, and 3 mg/kg) and quinpirole (0, 1, 3, and 9 mg/kg; indicated on *x*-axis). In *A*, the baseline stereotypy value was 1.0. *Black bars* show single agonist treatments. *White bars* show combined D_1 and D_2 agonist treatments. *B–D*, Induction of stereotypies by acute challenge with 6 mg/kg quinpirole (*B*), 6 mg/kg SKF 81297 (*C*), or SKF 81297 plus quinpirole (both 3 mg/kg) (*D*) after 1 week of either repeated cocaine or repeated saline treatment. Acutely, all combined agonist challenges, but not the single agonist challenges, induced significant levels of stereotypy (Kruskal–Wallis test; $H = 36.821$; $p < 0.001$, followed by Kolmogorov–Smirnov tests). Note the progressively increased levels of stereotypy induced by repeated cocaine treatment and the enhanced re-

Vanderschuren and Kalivas, 2000; Haile et al., 2001), with scores reaching values of ~6 on the stereotypy scale by day 7 (Fig. 1*B–D*). To test whether subsequent expression of this sensitized response required coactivation of D_1-class and D_2-class dopamine receptors, we administered D_1 and D_2 agonist challenges at dose levels within the ranges established in the drug-naive animals (3 mg/kg for each in the combined agonist treatments; for matching single agonist treatments, 6 mg/kg). Challenge with either SKF 81297 alone or quinpirole alone failed to induce intense stereotyped behaviors (Fig. 1*B,C*). In contrast, challenge with these D_1-class and D_2-class agonists combined elicited a significantly elevated motor response relative to that observed in saline-pretreated controls (Fig. 1*D*). The profile of behaviors induced by the drug combination included vigorous oral responses, such as persistent licking and nibbling. These results suggest that expression of the changes in motor behavior acquired as a result of repeated cocaine treatment require activation of both D_1-class and D_2-class dopamine receptors.
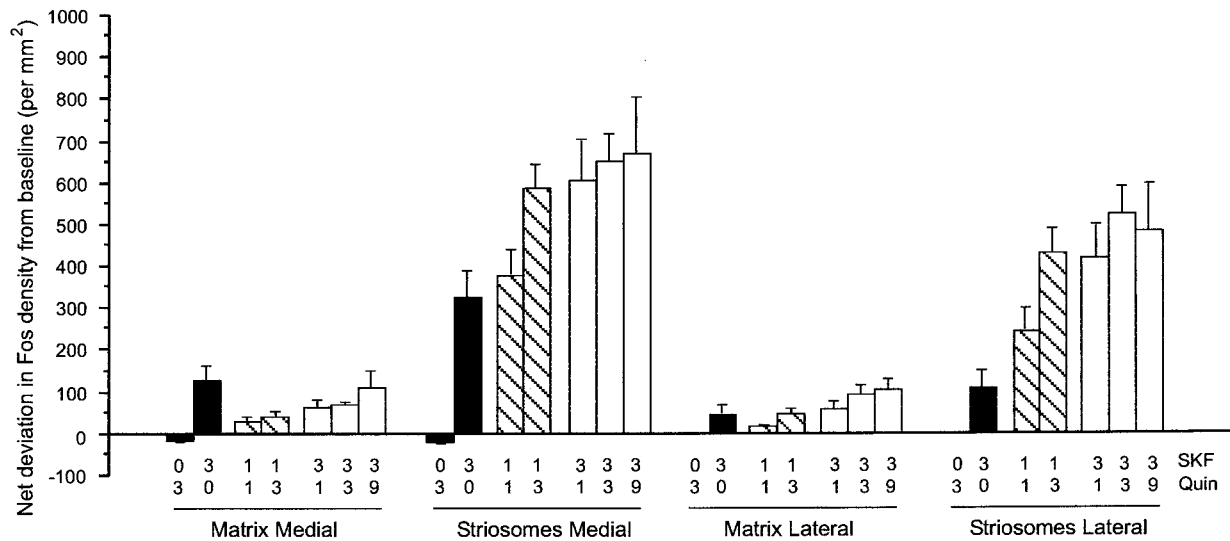
Coactivation of D_1-class and D_2-class dopamine receptors is required to induce differentially enhanced early gene activation in striosomes

The requirement for coactivation of D_1-class and D_2-class dopamine receptors in producing stereotypy in the drug-naive and cocaine-treated animals raised the possibility that D_1–D_2 synergism would also be essential to produce compartmental shifts in striatal early gene expression. We examined this possibility by performing immunohistochemistry to detect c-Fos and JunB together with the striosomal marker MOR1 in the same tissue sections. The results were clear-cut: in both groups, combined D_1–D_2 agonist challenge was required for expression of major shifts in the compartmental distribution of the early gene protein within the caudoputamen.

Figures 2 and 3 illustrate these findings for c-Fos. In the drug-naive rats, the D_2 agonist quinpirole slightly depressed the levels of Fos expression in the caudoputamen relative to control values (Figs. 2, 3*A*), whereas the D_1 agonist SKF 81297 produced a widespread expression of Fos, with only a twofold predominance in striosomes (Figs. 2, 3*B*) and slightly greater expression medially than laterally within the caudoputamen. A dramatic shift in the pattern of Fos distribution occurred in the caudoputamen after the combined D_1–D_2 agonist treatments. Fos expression was significantly increased in striosomes both medially and laterally (Figs. 2, 3*C,D*), but, in the matrix, the levels of Fos expression were mainly unaffected. The result was an elevation of ISMP values by several-fold (Fig. 4). The scatter in the data (Fig. 4) suggests that the D_1–D_2 combinations that included the high dose of SKF 81297 (3 mg/kg) activated the matrix more than the lower (1 mg/kg) doses without proportionally elevating striosomal activation, suggesting that high levels of D_1-class receptor stimulation did not further elevate the ISMP ratios beyond those obtained with low-dose D_1–D_2 combinations. Within the dose combinations we tested, any combination of the D_1 and D_2 agonists, but not treatment with either class of agonist given alone, producing a pronounced pattern of relative striosomal expression of Fos–Jun proteins.

←

Figure 2. Acute dopamine D_1-class and D_2-class receptor agonist treatments induce varying levels of early-gene expression in striosomes and matrix of the striatum. Values indicate net deviations from baseline levels of Fos expression in striosome and matrix compartments compared with values in saline-treated animals. Baseline values were as follows: medial matrix, 30.50 ± 9.51; medial striosomes, 39.60 ± 16.42; lateral matrix, 2.78 ± 1.32; and lateral striosomes, 3.81 ± 1.6. Of the single agonist challenges (*black bars*), only SKF 81297 induced significant levels of Fos expression in the striatum. Activation in the striosomes was much greater than activation in the matrix when increasing doses of quinpirole were combined with 1 mg/kg (*hatched bars*) or 3 mg/kg (*white bars*) SKF 81297. ANOVA indicated a significant high-order interaction of agonist × sector × compartment ($F_{(6,42)} = 3.113$; $p < 0.013$). *Quin,* Quinpirole; *SKF,* SKF 81297.

In the rats treated repeatedly with cocaine and then given a final challenge with either the D_1 or the D_2 agonist or the D_1–D_2 agonist combination, there was less striatal Fos expression than in the saline-pretreated control animals, reflecting a general down-regulation of excitability. Only the combined D_1–D_2 agonist treatment, however, produced significant alterations in the balance of Fos expression between striosomes and matrix (Fig. 5). With

single D_1-class or D_2-class agonist challenge after the intermittent cocaine treatment, the compartmental patterns of Fos expression (Figs. 6, $7A,A',B,B'$) and ISMP values (Fig. 5) were not different from those of rats repeatedly treated with saline. In contrast, when SKF 81297 and quinpirole were combined for the final agonist challenge after the cocaine exposure, there was a marked reduction of Fos expression in the matrix of the medial caudo-
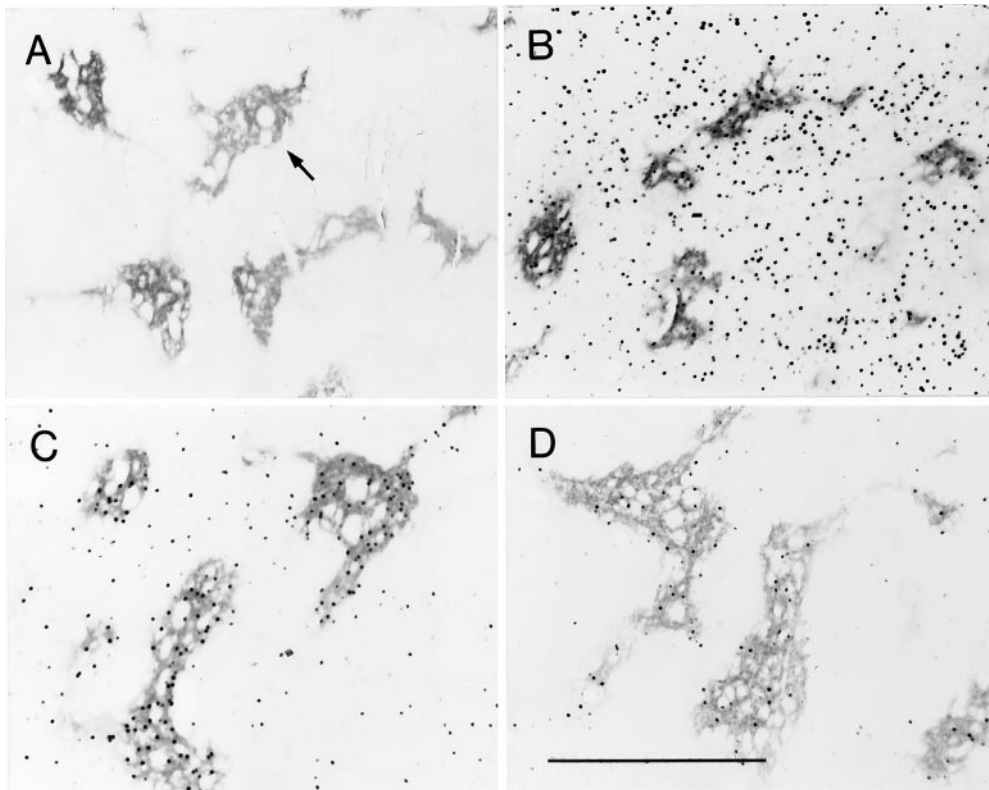
Figure 3. Dopamine D_1-class and D_2-class receptor agonists have differential effects on early-gene expression in the matrix and striosomes of the caudoputamen. Transverse coronal sections through the caudoputamen illustrating distributions of Fos-immunoreactive neurons in rats given acute challenges with the D_2 agonist quinpirole (3 mg/kg) (*A*), the D_1 agonist SKF 81297 (3 mg/kg) (*B*), and the D_1–D_2 agonist combinations of SKF 81297 plus quinpirole (3 mg/kg each) (*C*) or SKF 81297 plus quinpirole (1 mg/kg each) (*D*). Note that, in the animals given combined agonist challenge (*C, D*), clusters of Fos-positive nuclei are concentrated in striosomes, marked by strong μ-opioid receptor immunoreactivity. *Arrow* in *A* points to a μ-opioid receptor-positive striosome. Scale bar, 0.5 mm.
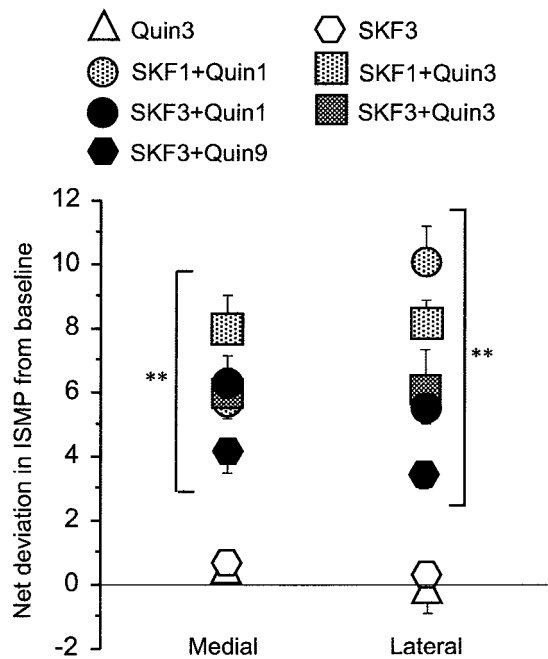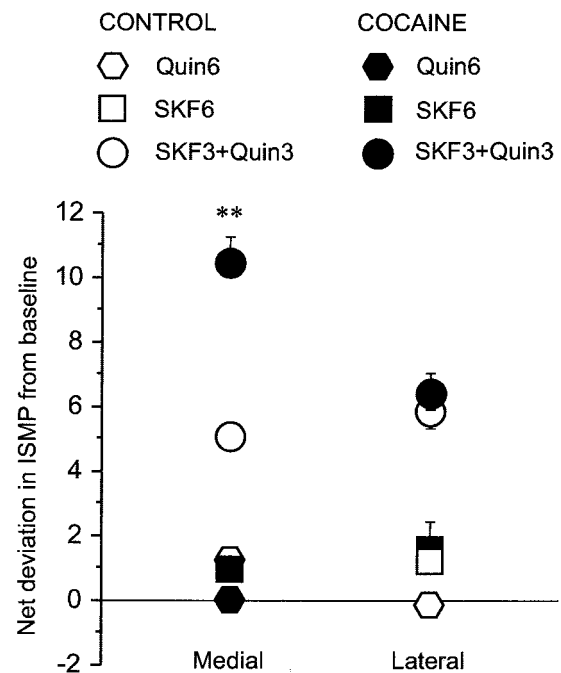
Figure 4. Indices of striosome-to-matrix predominance of early-gene activation (ISMP values) are increased by acute combinations of D_1-class (SKF 81297) and D_2-class (quinpirole) dopamine receptor agonist treatments. Values indicate deviations from baseline ISMP values. These were 1.30 ± 0.21 and 1.69 ± 0.98 for the medial and lateral sectors of the caudoputamen, respectively. Dosage (in milligrams per kilogram) is indicated with corresponding *symbols*. Single D_1 or D_2 agonist treatments did not elevate ISMP values significantly, whereas all combined D_1-class and D_2-class agonist challenges, regardless of dose levels, produced significant increases in ISMP values. ANOVA showed a significant effect of the interaction of agonist $\times$ sector ($F_{(6,42)} = 3.360$; $p < 0.009$), and only the combined treatments differed significantly from baseline (Newman–Keuls). **$p < 0.01$. *Quin*, Quinpirole; *SKF*, SKF 81297.

Figure 5. Repeated exposure to cocaine enhances striosome-predominant Fos expression in the medial caudoputamen in response to combined D_1-class and D_2-class dopamine receptor agonist challenge. The ISMP values shown indicate deviations from baseline ISMP values, which were 1.10 ± 0.16 and 0.66 ± 0.33 for the medial and lateral caudoputamen, respectively. Dosage is indicated with corresponding *symbols*. Combined, but not single, D_1-class or D_2-class agonist challenges elevated ISMP values significantly in control and cocaine-sensitized animals. In the latter group, the effect was potentiated in the medial sector of the caudoputamen. ANOVA indicated a significant drug $\times$ agonist $\times$ sector interaction ($F_{(2,36)} = 11.674$; $p < 0.001$). ISMP values differed significantly in response to D_1–D_2 challenge in saline-pretreated controls compared with cocaine-pretreated animals (Newman–Keuls). **$p < 0.01$. *Quin*, Quinpirole; *SKF*, SKF 81297.

putamen relative to control levels, and this reduction was much greater than that in striosomes (Figs. 6, 7C,C'). ISMP values therefore were significantly increased in the medial striatum (Fig. 5). Thus, combined activation of D_1-class and D_2-class receptors was necessary for the expression of the cocaine-induced changes in Fos–Jun protein distributions in the striatum. Qualitatively similar results were found for JunB (Fig. 7D,D').

### Enhanced activation of striosomes parallels enhanced behavioral stereotypy

To test whether the changes in stereotypy values and density values for Fos expression in the caudoputamen were correlated, we performed nonparametric versions of regression analysis. Figures 8 and 9 show scatter plots with all data points from both experiments with corresponding Spearman correlation values and significance levels. In our acute model (Fig. 8), early gene activation in the matrix medially was poorly correlated with the emergence of stereotypies, despite the fact that all acute challenges in which D_1 and D_2 agonists were coadministered increased Fos levels in this striatal compartment. Lateral activation in the matrix compartment did show a significant correlation. Early gene expression in striosomes correlated better with motor stereotypy than matrix activation, and both medial and lateral Spearman correlation values for striosomes were significant. Overall activation in the caudoputamen, regardless of compartmental effects, showed correlation values of 0.24 and 0.48 for medial and lateral sectors, respectively (scatter plots not shown). The best neural

estimate of motor stereotypy was given by the gene expression values in striosomes relative to expression values in the matrix, as defined by ISMP values. These exceeded the predictive power of the striosomal activation alone by 10% on average.

In the cocaine-pretreated animals (Fig. 9), decreases in Fos expression in the matrix occurred after combined D_1–D_2 agonist challenge relative to values in saline-pretreated animals, but, again, the density values for Fos expression in this compartment were not correlated with changes in behavior. Activation values for the striosomes were also decreased in rats given the combined D_1–D_2 challenge after exposure to cocaine, and the correlations between striosomal gene expression and behavior clearly exceeded those for the matrix compartment, reaching in both instances statistical significance. Overall levels of gene expression in the caudoputamen were not correlated with stereotypy (Spearman values were 0.06 and 0.30 for medial and lateral sectors, respectively; scatter plots not shown). Combined D_1–D_2 receptor agonist challenge after repeated cocaine treatment brought down levels of activation in the matrix to nearly baseline levels medially but only reduced activation levels in striosomes twofold. This differential downregulation produced significantly elevated ISMP values in the medial sector of the caudoputamen, and these ISMP values were better predictors of motor stereotypy than any other index of neural activation, including activation in the individual compartments. The Spearman rank correlation value for ISMP in
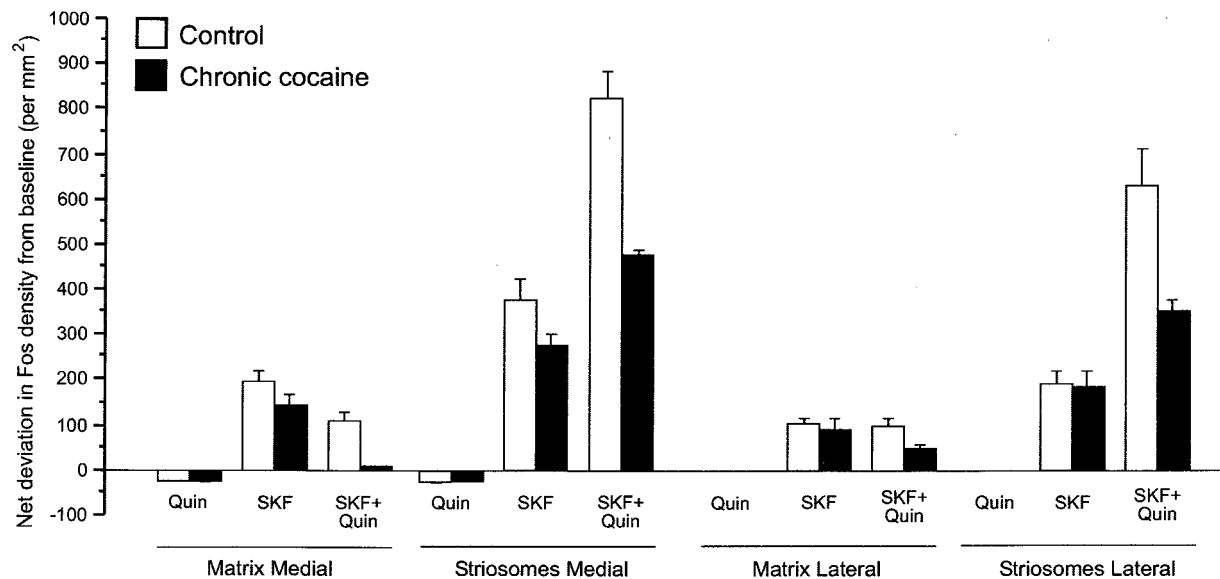
Figure 6. Challenges with combined D$_1$-class and D$_2$-class dopamine receptor agonists after repeated cocaine treatment show differential downregulation of Fos expression in the striatal matrix. Graphs show levels of Fos expression in striatal compartments after combined D$_1$-class and D$_2$-class dopamine receptor agonist challenges in rats that were saline pretreated (*white bars*) or cocaine pretreated (*black bars*). Values represent net deviations from baseline values, which were as follows: medial matrix, 29.32 ± 7.06; medial striosomes, 35.93 ± 13.12; lateral matrix, 4.60 ± 2.39; and lateral striosomes, 6.40 ± 3.73. Repeated exposure to cocaine (25 mg/kg, 7 d, twice per day) markedly downregulated Fos expression induced by all dopamine receptor agonist treatments. Note that, after cocaine plus combined D$_1$–D$_2$ challenge (3 mg/kg each), matrix Fos activation is almost nil in the caudoputamen medially, whereas net deviation from baseline in the striosomes is close to 500 cells/mm^2. ANOVA showed a significant interaction of drug × agonist × compartment ($F_{(2,36)}$ = 15.060; p < 0.001) and a nonsignificant interaction of drug × agonist × sector × compartment ($F_{(2,36)}$ = 0.485; p < 0.620).

medial caudoputamen was 0.76. This value (Fig. 9) was higher than that found for any of the analyses performed for the acutely treated animals (Fig. 8). In the chronic cocaine experiments, when calculations were performed for individual subgroups, similar correlations were observed between stereotypy and ISMP values in both the sensitized and naive subgroups of animals (data not shown). This analysis suggests that the relationship between relative striosome predominance and stereotypy does not depend on one particular preceding condition of drug exposure but, rather, bears on a general aspect of the regulation of the motor behavior.

DISCUSSION

The alterations in behavior and neural function induced by repeated exposure to psychomotor stimulants represent a striking example of prolonged, environmentally modulated neurobehavioral plasticity. These alterations can remain covert until an additional challenge with the drug occurs or until a stimulus contingently paired with the drug is presented. Our findings demonstrate that compartment-based neural alterations in the striatum are already evident during the sensitization regimen and that concurrent stimulation of D$_1$-class and D$_2$-class dopamine receptors is a necessary condition for uncovering them. Our findings further demonstrate that this neural plasticity is strongly correlated with the behavioral patterns induced by the D$_1$–D$_2$ challenges. Our observations suggest a link between the expression of repetitive behavior evoked by conjoint stimulation of D$_1$-class and D$_2$-class dopamine receptors and the expression of patterned alterations in relative activation of striosome-based and matrix-based basal ganglia circuits.

Requirement for synergisms between D$_1$-class and D$_2$-class dopamine receptors in evoking the expression of sensitized stereotypy

Many of the acute behavioral effects that are dependent on the activation of D$_2$-class dopamine receptors, including stereotypy and hyperlocomotion, are known to require simultaneous activation of D$_1$-class receptors. In the acute agonist treatment model that we used, combinations of D$_1$-class and D$_2$-class dopamine receptor agonist treatments were required to induce stereotypy, in accord with previous findings (Arnt et al., 1987; Clark and White, 1987; Walters et al., 1987; LaHoste et al., 1993; Waddington et al., 1995). Remarkably, after repeated cocaine treatments, the enhanced motor stereotypy that could be evoked by combined D$_1$–D$_2$ agonist challenge did not transfer to single-receptor class agonist challenge. Thus, despite the fact that sensitization had developed in the cocaine-pretreated animals, it remained covert even when animals were exposed to very high levels of either D$_1$-class or D$_2$-class dopamine receptor agonists alone.

Our observations are based on coordinated neural and behavioral measurements made directly after acute challenge or directly after postsensitization challenge. It is not known whether this requirement for combined D$_1$–D$_2$ dopamine receptor activation, evident at the end of the sensitizing period, holds also after extended periods of withdrawal. Enhanced stereotypy in response to D$_2$ receptor agonist challenge has been reported in one study in which rats were exposed to repeated cocaine treatment, followed by an intervening period of withdrawal (Ujike et al., 1990), which is thought to be critical for the development of some neural adaptations in dopamine and opioid neurotransmission. Our results suggest
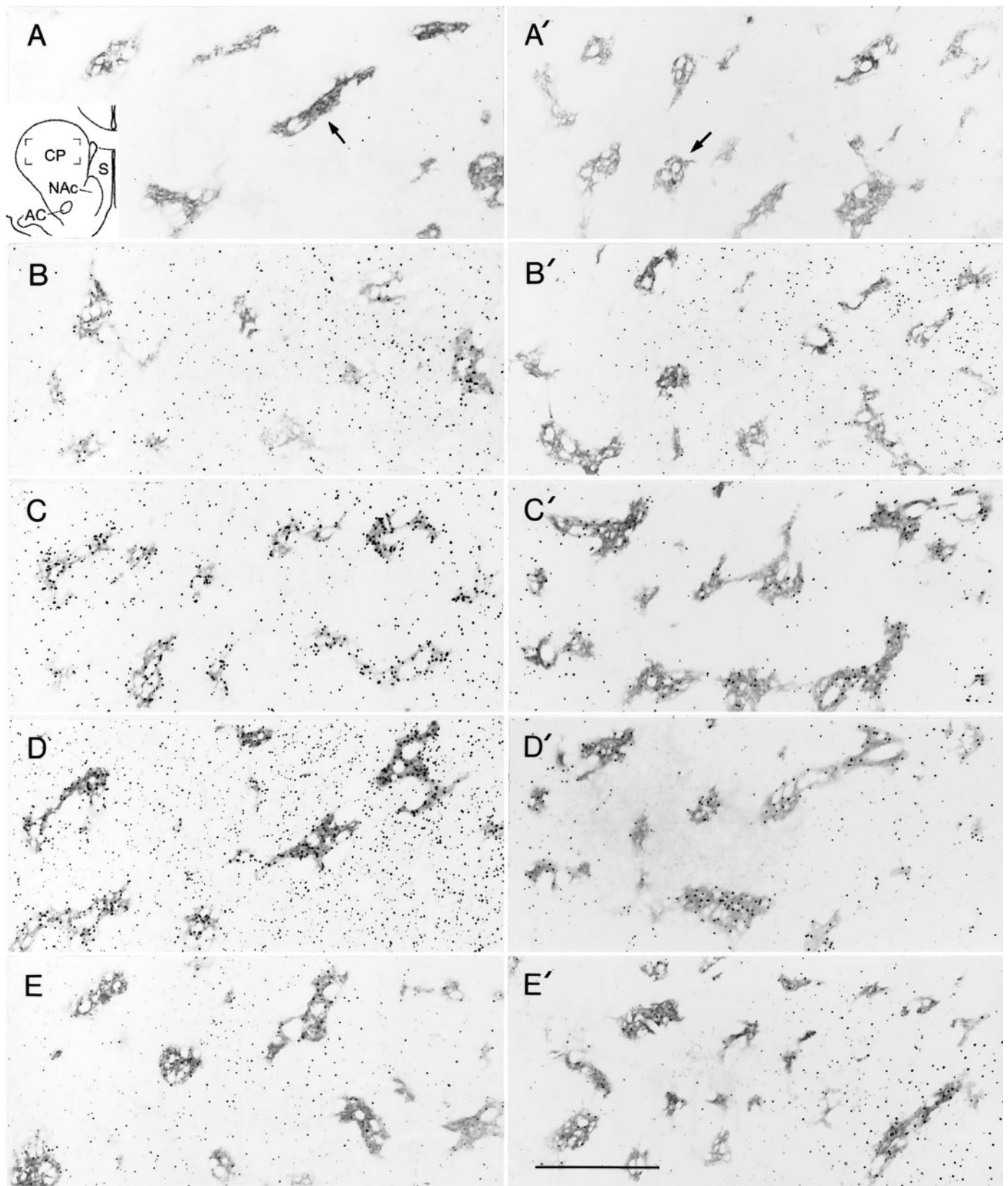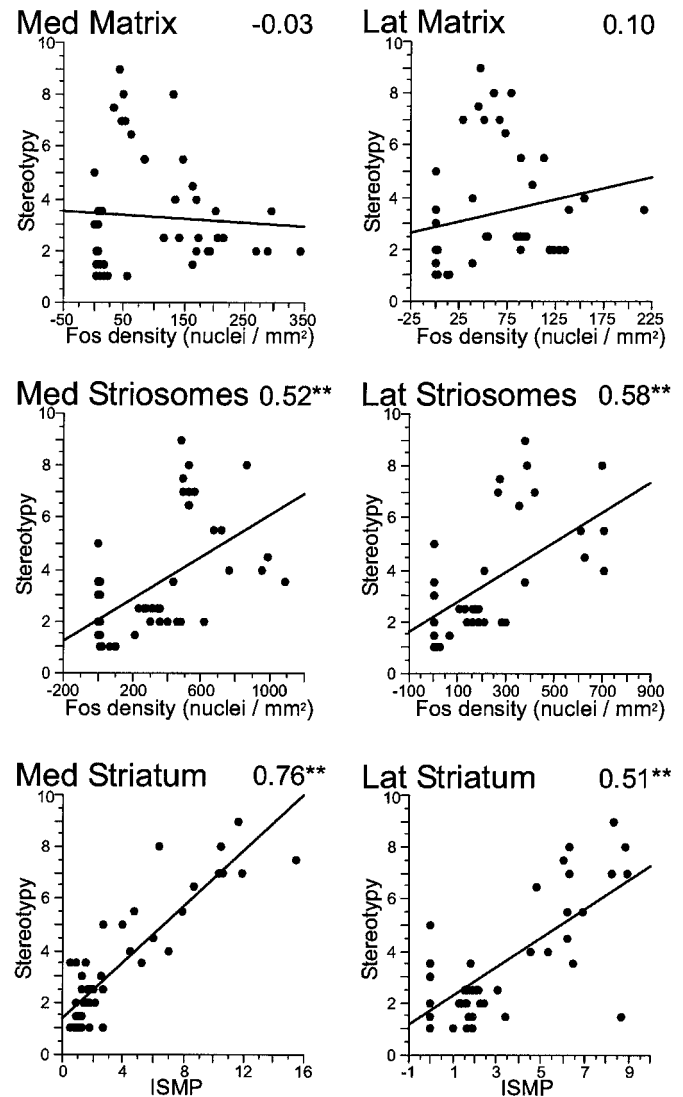
Figure 7. Immunohistochemical localization of c-Fos and JunB demonstrates synergistic effects of D$_1$-class and D$_2$-class receptors in striatum. Sections through the caudoputamen illustrating Fos (*A, A′, B, B′, C, C′*) or JunB (*D, D′, E, E′*) immunoreactivity in the striosomal and matrix compartments of the caudoputamen. Examples of striosomes are shown at *arrows* in *A* and *A′*. *A, A′*, A final challenge with the D$_2$-class agonist quinpirole (6 mg/kg) did not elicit Fos expression in rats previously exposed to saline (*A*) or to cocaine (25 mg/kg, 7 d, twice per day) (*A′*). *B, B′*, A final challenge with the D$_1$ agonist SKF 81297 (6 mg/kg) produced prominent, homogenous levels of Fos expression in saline-pretreated rats (*B*) and reduced levels in both compartments after repeated intermittent cocaine exposure (25 mg/kg, 7 d, twice per day) (*B′*). *C, C′, D, D′*, Combined D$_1$–D$_2$ agonist challenge with
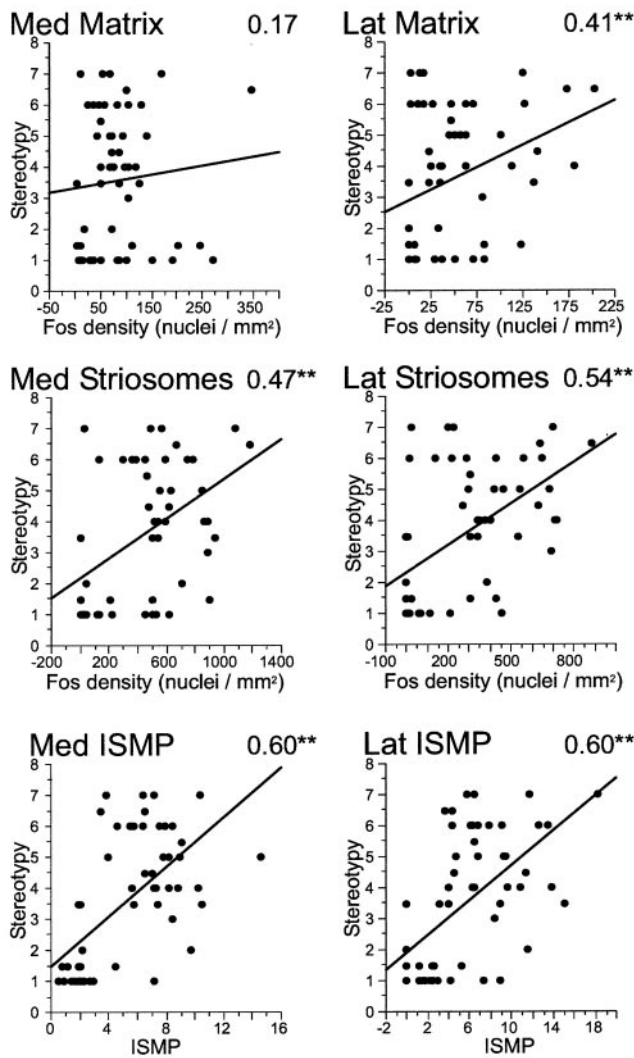
Figure 8. After acute treatments with combined D₁-class and D₂-class dopamine receptor agonists, the best predictor of motor stereotypy is the relative predominance of Fos expression in the striosomal compartment (ISMP value). Scatter plots representing individual data points for all animals included in the agonist–dose experiment and regression lines relating measures of Fos activation in the caudoputamen (*abscissa*) and stereotypy values (*ordinate*). The *number* at the *top right corner* of each plot is the Spearman correlation value (**p < 0.01). *Med Matrix*, Medial matrix; *Lat Matrix*, lateral matrix; *Med Striosomes*, medial striosomes; *Lat Striosomes*, lateral striosomes; *Med ISMP*, medial ISMP; *Lat ISMP*, lateral ISMP.

Figure 9. After repeated exposure to cocaine or saline, a strong correlation is found between stereotypy and the relative predominance of Fos expression in the striosomes (ISMP) induced by combined D₁–D₂ dopamine receptor agonist challenge. Scatter plots show individual data points for all animals included in the cocaine sensitization experiment and corresponding regression lines. *Top four plots* show data for Fos expression in medial (*Med*) and lateral (*Lat*) matrix and striosomes relative to stereotypy values, and *two bottom plots* show ISMP values for medial and lateral caudoputamen relative to stereotypy scores. The *number* at the *top right corner* of each plot represents the Spearman correlation value. **p < 0.01.

that whatever sensitization of D₁-class and D₂-class receptor function occurred as a result of the drug exposure given to the animals, agonist challenge of either one of these receptor systems was insufficient to evoke the expression of the latent, behavioral adaptations that took place during the sensitization phase.

Requirement for D₁–D₂ dopamine receptor synergisms in evoking the expression of striosome-predominant early gene distributions

Based on our previous finding that stereotypy induced by psychomotor stimulants is highly correlated with striosome-pre-

←

dominant early-gene expression evoked by the same treatments, we tested whether these neural changes, like the behavioral changes, developed during drug sensitization and required concurrent activation of D$_1$-class and D$_2$-class dopamine receptors. They did. After repeated cocaine treatment, the early-gene responses induced by single D$_1$-class or D$_2$-class agonist challenge were downregulated relative to those in the striatum of drug-naive animals, but there was no difference in the relative response of neurons in striosomes and matrix. In contrast, in animals challenged with D$_1$–D$_2$ agonist combinations, there was a significantly greater downregulation in the matrix than in striosomes, especially medially, leading to greater relative activation in the striosomal compartment. The degree of stereotypy induced by the combined D$_1$–D$_2$ agonist challenge showed poor correlation with the matrix downregulation or with estimates of overall striatal gene expression but strong correlation with striosomal activation and even higher correlation with the relative striosomal predominance of gene expression in the medial striatum.

A similar dependency on combined activation of D$_1$-class and D$_2$-class dopamine receptors held for the full range of acute single agonist treatments that we used to treat drug-naive rats, extending previous observations on the regulation of early response genes by dopamine receptor agonists (LaHoste et al., 1993; Wirtshafter and Asin, 1994; Wirtshafter et al., 1997). We did not observe dose dependency for this effect, but lower doses of D$_1$ and D$_2$ agonists than those used in our experiments might show dose dependency just as apomorphine, a mixed D$_1$–D$_2$ agonist, increases both stereotypy and striosomal predominance in a dose-dependent manner (Canales and Graybiel, 2000).

Correlations between stereotypy and striosome-predominant gene activation in the striatum

The degree of stereotypy induced by direct dopamine receptor agonists in our experiments was correlated with a relative enhancement of striosomal early-gene activation not only in cocaine-sensitized animals but also in drug-naive animals. In naive animals, neuronal activation in the matrix and the striosomes increased in parallel with stereotypy, whereas activation in both compartments was reduced after repeated cocaine exposure, a treatment that conversely produced higher levels of stereotypy. However, levels of activity in the striosomes alone showed significant correlations with the stereotypy ratings, which is in accord with previous findings (Canales and Graybiel, 2000). The results we report here suggest that these behavioral and neural responses are regulated either in parallel or in common by a mechanism involving concomitant activation of D$_1$-class and D$_2$-class dopamine receptors. Such synergistic D$_1$–D$_2$ effects on activation of striosomes and matrix may give clues to the specific mechanisms underlying the behavioral expression of sensitized stereotypies. For example, the cholinergic and nitric oxide synthase-containing interneurons of the striatum have been suggested to act in the induction of the striosome-predominant pattern of gene expression in response to drug challenge (Graybiel et al., 2000; Svenningsson et al., 2000; Saka et al., 2002), as has upregulation of dynorphin in striatal neurons and enhanced corticostriatal transmission (Moratalla et al., 1996; Canales and Graybiel, 2000).

In rats exposed to repeated cocaine treatment, combined D$_1$–D$_2$ agonist challenge, but not single agonist treatments, reproduced the main phenotypes of cocaine sensitization: increased stereotypy after challenge and increased striosome-to-matrix neuronal activation. Some important differences between D$_1$–D$_2$ agonist challenge and cocaine challenge were also noted. In cocaine-sensitized animals, a final challenge with cocaine produces an enhanced pattern of striosomal activation in the lateral caudoputamen (Canales and Graybiel, 2000), whereas with combined D$_1$–D$_2$ agonist challenge, the striosomal enhancement was mainly medial. Activity in striosomes was decreased by combined D$_1$–D$_2$ challenge, but increased by cocaine challenge, after a sensitizing regimen of cocaine exposure. The combined D$_1$–D$_2$ treatment elicited effects reminiscent of amphetamine challenge after repeated amphetamine exposure, reducing intrinsic striosomal activation yet increasing relative striosomal activation both medially and laterally in the caudoputamen attributable to even greater reduction in the matrix. Acute apomorphine treatment, which directly stimulates D$_1$ and D$_2$ receptors, also enhances relative striosomal activity medially (Canales and Graybiel, 2000). Such topographic differences might relate to the fact that the motor responses emitted in response to different psychomotor stimulants and D$_1$–D$_2$ agonists have different specific patterns, despite sharing common characteristics of repetitiveness and stereotypy. The medial pattern of striosome predominance is also found in rodent models of parkinsonism, in which rats with 6-hydroxydopamine-induced lesions of the nigrostriatal pathway are chronically exposed to levodopa (Cenci et al., 1999) or are given apomorphine (Saka et al., 1999) or combined D$_1$–D$_2$ dopamine receptor agonist treatment (Paul et al., 1992). Jonathan M. Brotchie (University of Manchester, Manchester, UK) (personal communication) has reported such striosome-enhanced patterns of preproenkephalin in the brains of dyskinetic Parkinson's disease patients, and a similar enhancement of striosomal prodynorphin mRNA was noted in a case report of a cocaine addict (Hurd and Herkenham, 1993).

Our findings suggest a bridge between the effects of direct dopamine receptor agonists and the effects of drugs of abuse. A key factor for both is combined activation of D$_1$-class and D$_2$-class dopamine receptors. Such conjoint dopamine receptor stimulation is thought to lead to activation of the direct pathway and inhibition of the indirect pathway leading out of the striatum (Gerfen and Wilson, 1996) and thus to activation of the motor thalamus and frontal neocortex through basal ganglia–thalamocortical loop pathways. Repeated overactivation of these striatocortical loops may result in effects akin to "kindling" of the frontal cortex that could favor the emergence of repetitive behaviors in the absence of external cues for adaptive movement. A close relationship between stereotypy and striosome-predominant neuronal activity could, in particular, reflect a persistent differential recruitment of striosome-based cortico-basal ganglia circuits involving medial and orbital frontal cortex (Gerfen, 1984; Donoghue and Herkenham, 1986; Ragsdale and Graybiel, 1990; Eblen and Graybiel, 1995; Volkow and Fowler, 2000; Goldstein et al., 2001). Such persistent pathway selection could trigger the compulsion to repeat the same few behaviors at the expense of the initiation of adaptive, switching responses requiring threshold activation of matrix-based basal ganglia circuits (Peterson et al., 1998; Graybiel and Rauch, 2000; Leckman et al., 2001).

Another possibility is that the presumably inhibitory striatonigral projection could have a homeostatic function to decrease dopaminergic stimulation of the striatum and, in turn, to decrease increased repetitions of elicited actions. Such an adaptive control mechanism would not, however, be expected to be completely effective if the source of dopaminergic stimulation is from exogenous sources rather than from intrinsic activity of the part of the substantia nigra pars compacta interconnected with the striosomal system. The ineffectiveness of such a striosome-based homeostatic

mechanism could contribute to the development of stereotypy in behavioral sensitization, as studied here, as well as levodopa-induced dyskinesias, drug-induced compulsions, Tourette syndrome, and obsessive-compulsive spectrum disorders.

REFERENCES

Antony MM, Downie F, Swinson RP (1988) Diagnostic issues and epidemiology in obsessive-compulsive disorder. In: Obsessive-compulsive disorder: theory, research and treatment (Swinson RP, Antony MM, Rachman S, Richter MA, eds). Oxford: Guildford.

Arnt J (1985) Antistereotypic effects of dopamine D-1 and D-2 antagonists after intrastriatal injection in rats. Pharmacological and regional specificity Naunyn Schmiedebergs Arch Pharmacol 330:97–104.

Arnt J, Hyttel J, Perregaard J (1987) Dopamine D-1 receptor agonists combined with the selective D-2 agonist quinpirole facilitate the expression of oral stereotyped behaviour in rats. Eur J Pharmacol 133:137–145.

Arnt J, Bogeso KP, Hyttel J, Meier E (1988) Relative dopamine D1 and D2 receptor affinity and efficacy determine whether dopamine agonists induce hyperactivity or oral stereotypy in rats. Pharmacol Toxicol 62:121–130.

Berke JD, Hyman SE (2000) Addiction, dopamine, and the molecular mechanisms of memory. Neuron 25:515–532.

Canales JJ, Graybiel AM (2000) A measure of striatal function predicts motor stereotypy. Nat Neurosci 3:377–383.

Canales JJ, Iversen SD (1998) Behavioural topography in the striatum: differential effects of quinpirole and D-amphetamine microinjections. Eur J Pharmacol 362:111–119.

Cenci MA, Tranberg A, Andersson M, Hilbertson A (1999) Changes in the regional and compartmental distribution of FosB- and JunB-like immunoreactivity induced in the dopamine-denervated rat striatum by acute or chronic L-dopa treatment. Neuroscience 94:515–527.

Clark D, White FJ (1987) Rev: D1 dopamine receptor—the search for a function: a critical evaluation of the D1/D2 dopamine receptor classification and its functional implications. Synapse 1:347–388.

Creese I, Iversen SD (1972) Amphetamine response in rat after dopamine neurone destruction. Nat New Biol 238:247–248.

Cummings JL (1995) Anatomic and behavioral aspects of frontal-subcortical circuits. Ann NY Acad Sci 769:1–13.

Donoghue JP, Herkenham M (1986) Neostriatal projections from individual cortical fields conform to histochemically distinct striatal compartments in the rat. Brain Res 365:397–403.

Eblen F, Graybiel AM (1995) Highly restricted origin of prefrontal cortical inputs to striosomes in the macaque monkey. J Neurosci 15:5999–6013.

Fibiger HC, Fibiger HP, Zis AP (1973) Attenuation of amphetamine-induced motor stimulation and stereotypy by 6-hydroxydopamine in the rat. Br J Pharmacol 47:683–692.

Gerfen CR (1984) The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems. Nature 311:461–464.

Gerfen CR, Wilson CJ (1996) The basal ganglia. In: Handbook of chemical neuroanatomy (Swanson LW, Björklund A, Hökfelt T, eds), pp 371–468. Amsterdam: Elsevier.

Goldstein RZ, Volkow ND, Wang GJ, Fowler JS, Rajaram S (2001) Addiction changes orbitofrontal gyrus function: involvement in response inhibition. NeuroReport 12:2595–2599.

Graybiel AM, Canales JJ (2001) The neurobiology of repetitive behaviors: clues to the neurobiology of Tourette syndrome. In: Tourette syndrome (Cohen D, Goetz C, Jankovic J, eds), pp 123–131. Philadelphia: Williams and Wilkins.

Graybiel AM, Rauch SL (2000) Toward a neurobiology of obsessive-compulsive disorder. Neuron 28:343–347.

Graybiel AM, Canales JJ, Capper-Loup C (2000) Levodopa-induced dyskinesias and dopamine-dependent stereotypies: a new hypothesis. Trends Neurosci 23:S71–S77.

Haile CN, Hiroi N, Nestler EJ, Kosten TA (2001) Differential behavioral responses to cocaine are associated with dynamics of mesolimbic dopamine proteins in Lewis and Fischer 344 rats. Synapse 41:179–190.

Hurd YL, Herkenham M (1993) Molecular alterations in the neostriatum of human cocaine addicts. Synapse 13:357–369.

Iorio LC, Barnett A, Leitz FM, Houser VP, Korduba CA (1983) SCH23390 a potential benzazepine antipsychotic with unique interactions on dopaminergic systems. J Pharmacol Exp Ther 226:462–468.

Jung BJ, Dawson Jr R, Sealey SA, Peris J (1999) Endogenous GABA release is reduced in the striatum of cocaine-sensitized rats. Synapse 34:103–110.

Kalivas PW, Stewart J (1991) Dopamine transmission in drug- and stress-induced behavioral sensitization. Brain Res Brain Res Rev 16:223–244.

Karler R, Calder LD, Thai LH, Bedingfield JB (1995) The dopaminergic, glutamatergic, GABAergic bases for the action of amphetamine and cocaine. Brain Res 671:100–104.

LaHoste GJ, Yu J, Marshall JF (1993) Striatal Fos expression is indic-

ative of dopamine D1/D2 synergism and receptor supersensitivity. Proc Natl Acad Sci USA 90:7451–7455.

Leckman JF, Cohen DJ, Goetz CG, Jankovic J (2001) Tourette syndrome: pieces of the puzzle. Adv Neurol 85:369–390.

Manschreck TC (1986) Motor abnormalities in schizophrenia. In: The neurobiology of schizophrenia (Nasrallah HA, Weinberger DR, eds). Amsterdam: Elsevier.

Moratalla R, Elibol B, Vallejo M, Graybiel AM (1996) Network-level changes in expression of inducible Fos-Jun proteins in the striatum during chronic cocaine treatment and withdrawal. Neuron 17:147–156.

Nestler EJ, Hyman SE, Malenka RC (2001) Molecular basis of neuropharmacology: a foundation for clinical neuroscience. New York: McGraw-Hill.

Paul ML, Graybiel AM, David J-C, Robertson HA (1992) D1-like and D2-like dopamine receptors synergistically activate rotation and c-fos expression in the dopamine-depleted striatum in a rat model of Parkinson's disease. J Neurosci 12:3729–3742.

Peterson BS, Skudlarski P, Anderson AW, Zhang H, Gatenby JC, Lacadie CM, Leckman JF, Gore JC (1998) A functional magnetic resonance imaging study of tic suppression in Tourette syndrome. Arch Gen Psychiatry 55:326–333.

Ragsdale Jr CW, Graybiel AM (1990) A simple ordering of neocortical areas established by the compartmental organization of their striatal projections. Proc Natl Acad Sci USA 87:6196–6199.

Richter A, Ebert U, Nobrega JN, Vallbacka JJ, Fedrowitz M, Loscher W (1999) Immunohistochemical and neurochemical studies on nigral and striatal functions in the circling (ci) rat, a genetic animal model with spontaneous rotational behavior. Neuroscience 89:461–471.

Ridley RM (1994) The psychology of perseverative and stereotyped behaviour. Prog Neurobiol 44:221–231.

Robinson TE, Becker JB (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res 396:157–198.

Robinson TE, Browman KE, Crombag HS, Badiani A (1998) Modulation of the induction or expression of psychostimulant sensitization by the circumstances surrounding drug administration. Neurosci Biobehav Rev 22:347–354.

Rosenberg DR, Dick EL, O'Hearn KM, Sweeney JA (1997) Response-inhibition deficits in obsessive-compulsive disorder: an indicator of dysfunction in frontostriatal circuits. J Psychiatry Neurosci 22:29–38.

Saka E, Elibol B, Erdem S, Dalkara T (1999) Compartmental changes in expression of c-Fos and FosB proteins in intact and dopamine-depleted striatum after chronic apomorphine treatment. Brain Res 825:104–114.

Saka E, Iadarola M, Fitzgerald DJ, Graybiel AM (2002) Local circuit neurons in the striatum regulate neural and behavioral responses to dopaminergic stimulation. Proc Natl Acad Sci USA 99:9004–9009.

Svenningsson P, Fredholm BB, Bloch B, Le Moine C (2000) Co-stimulation of D(1)/D(5) and D(2) dopamine receptors leads to an increase in c-fos messenger RNA in cholinergic interneurons and a redistribution of c-fos messenger RNA in striatal projection neurons. Neuroscience 98:749–757.

Turski L, Schwarz M, Sontag KH (1984) Stereotypy, locomotor and cataleptic effects produced by drugs influencing dopaminergic systems in a mutant strain of Wistar rats: a genuine model of basal ganglia dysfunction? Behav Brain Res 12:29–37.

Ujike H, Akiyama K, Otsuki S (1990) D-2 but not D-1 dopamine agonists produce augmented behavioral response in rats after subchronic treatment with methamphetamine or cocaine. Psychopharmacology 102:459–464.

Vanderschuren LJ, Kalivas PW (2000) Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology 151:99–120.

Volkow ND, Fowler JS (2000) Addiction, a disease of compulsion and drive: involvement of the orbitofrontal cortex. Cereb Cortex 10:318–325.

Waddington JL, Daly SA, Downes RP, Deveney AM, McCauley PG, O'Boyle KM (1995) Behavioural pharmacology of "D-1-like" dopamine receptors: further subtyping, new pharmacological probes and interactions with "D-2-like" receptors. Prog Neuropsychopharmacol Biol Psychiatry 19:811–831.

Walters JR, Bergstrom DA, Carlson JH, Chase TN, Braun AR (1987) D1 dopamine receptor activation required for postsynaptic expression of D2 agonist effects. Science 236:719–722.

White IM, Doubles L, Rebec GV (1998) Cocaine-induced activation of striatal neurons during focused stereotypy in rats. Brain Res 810:146–152.

Wirtshafter D, Asin KE (1994) Interactive effects of stimulation of D1 and D2 dopamine receptors on fos-like immunoreactivity in the normosensitive rat striatum. Brain Res Bull 35:85–91.

Wirtshafter D, Schardt G, Asin K (1997) Compartmentally specific effects of quinpirole on the striatal Fos expression induced by stimulation of D1-dopamine receptors in intact rats. Brain Res 771:272–277.